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VANADIUM AND EXPERIMENTAL CARIES. VII. ACTION OF VANADIUM ON THE DEVELOPMENT OF LACTOBACILLUS ACIDOPHILUS AND STREPTOCOCCUS LACTIS

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VANADIUM AND EXPERIMENTAL CARIES
[Vanadio E Carie Sperimentale]

VII. Action of Vanadium on the Development of Lactobacillus acidophilus and Streptococcus lactis
[VII - Azione del Vanadio sullo sviluppo del Lactobacillus acidophilus e dello Streptococcus lactis.]

by

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Study of the most diverse substances that have been tested over the years for their carioprophylactic activity on animals and on man himself, is encumbered by technical and applicative difficulties owing to the fact that the true causes of the carious process remain unknown.

Consequently it sometimes happens that we succeed in reducing carious affections using a certain procedure without being able to explain exhaustively the biological process by means of which this result has been obtained. This is so even if we take into

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account all the most accredited theories and analyze our results in these terms.

One of the most important points regarding the role of microbial components in the determination of the carious process has been decisively elucidated on the basis of experiments carried out first by Blayney and then by Orland et al., who were able to establish that carious processes do not develop in experimental animals subjected to a cariogenic diet but bred in a germfree environment.

These data have demonstrated that in order for the carious process to set in, genetic and nutritional factors do not suffice: the presence of germs in the oral cavity is also absolutely necessary.

The experimental researches tending to demonstrate such a relationship of cause and effect confirm the prevalence of lacto - bacilli and streptococchi in the development of carious lesions (Lewis, Snyder, Permar, Hartman, Kitchin). On the heels of these experiments tests have been elaborated that are based on a count of the colonies that can be isolated from the saliva of different subjects. These tests are designed to evaluate the carious activity in a given individual in order to adopt in time the necessary measures to stem the advance of carious lesions.

Numerous authors (Bunting, Lewis, Snyder, Permar) have proven the exactitude of these tests by demonstrating for example that the degree of carious activity is directly proportional to the number of lactobacilli present in the saliva.

In support of the view that lactobacilli play a role in caries is cited their constant presence in the oral cavity and, especially, the ability of these germs to ferment the simpler sugars to produce

acids, in particular lactic acid, which are thought to enter into the determination of caries in various ways; and according to more recent views these acids behave like true catalysts, mobilizing the calcium salts of the hard tissues and thus causing the formation of cavities that could lead to complications.

On the analogy of what has already been done for fluorine and other carioprophylactic agents (Dena, Arnold, Jay, Ruth), the antibacterial activity of which has frequently been tested, decided to test vanadium on germs too, choosing from among the strains most grequently isolated from the oral cavity: lactobacillus acidophilus and Streptococcus lactis.

The experiments were donducted by first testing the ability of the two strains to develop in the presence of solutions with different concentrations of vanadium; then we examined both strains for their ability to produce lactic acid when solutions of vanadium were added to the culture medium.

It is obvious that the development and metabolism conditions of the two bacterial strains, studied though they were under optimal experimental conditions <u>in vitro</u>, could have been carried out on strains isolated from hansters and, within the limits imposed by practical considerations, in culture media as close as possible to the diets utilized; this is what we intend to do in experiments that have already been programmed.

1. Development Tests for Lactobacillus acidophilus and Streptococcus lactis in the presence of Vanadium.

We used a strain of streptococcus lactis and a strain of lactobacillus acidophilus from the collection of the Veterinary Laboratories of the I.S.S.

Obviously all the experiments were carried out under sterile conditions. For each strain we prepared 6 series of 5 test-tubes, each containing 9 ml of skim-milk with a slightly acid pH; before insemination, to each series we added 1 ml of a vanadium pentoxide solution in respective concentrations of 20, 40, 100, 200, 400 and 20,000 ppm ; in each test-tube of the series we inseminated 0.5 ml of a pure culture of lactobacillus acidophilus and streptococcus lactis, respectively.

Five days of incubation resulted in an abundant increase and curd of milk in the test tubes to which had been added 20, 40, 100, 200 and 400 ppm of V_2O_5, both in the case of lactobacillus acidophilus and streptococcus lactis, while in the sixth series the medium remained uncoagulated; only after seven days did we observe a partial curdling in three out of five test tubes, both for streptococcus lactis and lactobacillus acidophilus. The development of the controls was normal and analogous to the series prepared with increasing concentrations of V_2O_5 from 20 to 400 ppm.

Hence, it is only in high doses that vanadium exercises a relative bacteriostatic action, as is shown by the test made with 20,000 ppm and despite the optimal conditions of the culture in vitro.

2. Production of Lactic Acid by Lactobacillus acidophilus and Streptococcus lactis in the presence of Vanadium.

The development tests for lactobacillus acidophilus and streptococcus lactis demonstrated that the addition of various concentrations of vanadium to the culture medium did not inhibit the development of the two strains. It remained to be seen,

however, whether the typical metabolic property of the two strains, namely, their ability to produce lactic acid, would undergo any change.

To this end we carried out appropriate experiments using the two bacterial strains that had already been tested.

We prepared ten series of five 250 ml flasks containing 100 ml of skim milk with a neutral pH. To each series we added 1 ml of solutions of V_2O_5 in respective concentrations of 10, 20, 40 and 200 ppm; one series was kept as the control. In five series of flasks we inseminated 1 ml of a pure culture of lactobacillus and in the other five series, 1 ml of a pure culture of streptococcus.

The flasks were thermostated at 30°C for 6 days and then in the culture medium we determined the degree of acidity (Soxhlet - Henkel degree) by means of titration with N/4 soda in the presence of 1% alcoholic phenolphthalein.. The degrees of acidity of the different series expressed in lactic acid by application of the well known correction factor (0.0225) did not disclose any significant differences when we subjected them to a mathematico-statistical comparison.

Hence, the addition to the culture medium of solutions of vanadium in different concentrations does not modify the ability of either lactobacillus acidophilis or streptococcus lactis to produce lactic acid.

The results of our experiments in vitro on strains of streptococcus lactis and lactobacillus acidophilus enable us to exclude the possibility that vanadium, as caries inhibiting agent, acts by depressing the development of the supposedly cariogenic flora of the oral cavity.

By now the role that streptococcus lactis and lactobacillus acidophilus play in the etiopathogenetic mechanism of caries is well known.

Orland et al. have found an absolute absence of carious lesions in mice bred in a germfree environment and have demonstrated experimentally that in hamsters the association of lactobacilli and streptococchi develops carious lesions; many other researchers, taking up this working hypothesis, have confirmed that these germs can reproduce experimentally in the hamster lesions similar to caries (Fitzgerald, Hurst).

Finally, it should be recalled that both streptococcus lactis and lactobacillus acidophilus have often been isolated by experimenters from human carious lesions and for this very reason have already been taken into consideration by Ruth, Itzhac and Sulitzeao in order to explain the caries inhibiting mechanism of another trace element: fluorine. Flor fluorine too, however, the question is controversial and remains in the realm of hypotheses: indeed, while some have found a decrease in the number of germs in the saliva after administration of fluorinated water, others have not detected any correlation between the number of lactobacilli and the caries inhibiting effect.

If, however, we consider that the germs act in well-delineated zones, namely, at the level of the so-called bacterial plaques adhering to the surface of the teeth, then the number of germs present there, i.e., to count more or less of them, is not so important as the ability of the germs themselves to acidify the medium. For this reason in our tests we investigated the acidogenic

activity of the experimental strains to see whether vanadium could act not only by inhibiting an increase in their number but also by modifying the enzymatic activities that permit the production of lactic acid.

That these hypotheses are unfounded is demonstrated by the fact that both lactobacillus and streptococcus, when placed in a culture medium to which different doses of vanadium have been added, develop normally and, as in the case of the controls, acidify the culture medium, which, consisting as it does of skim milk, curdles. That the curdling is caused by acidification and not by alkalinization of the medium is proved by the titration with N/4 soda of the lactic acid produced by the germs in the presence as well as the absence of vanadium.

Consequently, taking into account the experimental conditions adopted, the results of our tests enable us to say that vanadium, in the context of prophylaxis of caries artificially induced in hamsters by administration of a cariogenic diet, exercises its beneficial action not through any influence on the microbial flora present in the oral cavity but, as is apparent for the rest, from spectrochemical experiments (see note VI, Cechetti et al.), on an exclusively organistic basis; in other words, it enters into the chemical structure of the tooth.

SUMMARY

The action of solutions with different contents of V on the development and on the production of lactic acid of cultures of lactobacillus acidophilus and streptococcus lactis was investigated by the authors.

The authors have established that there are no differences between the culture and the controls except for a relative bacteriostatic action (but only with very high doses of V).

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